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SYSTEMATIC REVIEW AND META-ANALYSIS

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Potential Role of Circulating MiR-21 in the Diagnosis and Prognosis of Digestive System Cancer

A Systematic Review and Meta-Analysis

Chengqiang Yin, MS, Xiaoying Zhou, PhD, Yini Dang, PhD, Jin Yan, PhD, and Guoxin Zhang, MD, PhD

Abstract: Recent evidences indicate that circulating microRNAs (miRNAs) exhibit aberrant expression in the plasma of patients suffering from cancer compared to normal individuals, suggesting that it may be a useful noninvasion diagnostic method. MiR-21 plays crucial roles in carcinogenesis and can be served as a biomarker for the detection of various cancers. Therefore, the aim of this meta-analysis is to assess the potential role of miR-21 for digestive system cancer.

By searching the PubMed, Embase, and Web of Science for publications concerning the diagnostic value of miR-21 for digestive system cancer, total of 23 publications were included in this meta-analysis. Receiver operating characteristic curves (ROC) were used to check the overall test performance. For prognostic meta-analysis, pooled hazard ratios (HRs) of circulating miR-21 for survival were calculated.

Totally 23 eligible publications were included in this meta-analysis (15 articles for diagnosis and 8 articles for prognosis). For diagnostic meta-analysis, the summary estimates revealed that the pooled sensitivity and specificity were 0.76(95% CI = 0.70–0.82) and 0.84 (95% CI = 0.78–0.89). Besides, the area under the summary ROC curve (AUC) is 0.87. For prognostic meta-analysis, the pooled HR of higher miR-21 expression in circulation was 1.94 (95% CI = 0.99–3.82, $P = 0.055$), which indicated higher miR-21 expression could be likely to predict poorer survival in digestive system cancer. The subgroup analysis implied the higher expression of miR-21 was correlated with worse overall survival in the Asian population in digestive system cancer (HR = 2.41, 95% CI = 1.21–4.77, $P = 0.012$).

The current evidence suggests circulating miR-21 may be suitable to be a diagnostic and prognostic biomarker for digestive system cancer in the Asians.

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From the Department of Gastroenterology (CQY, XYZ, YND, JY,GXZ), First Affiliated Hospital of Nanjing Medical University; Department of Gastroenterology (CQY), Mingde Hospital Affiliated with Nanjing Medical University; First Clinical Medical College of Nanjing Medical University, Nanjing 210029, China.
Correspondence: Guoxin Zhang, Department of Gastroenterology, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China (e-mail: guoxinz@njmu.edu.cn).
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Abbreviations: AUC = area under the summary ROC curve, CRC = colorectal cancer, DE = data-extrapolated, DOR = diagnostic odds ratio, DOR = diagnostic odds ratio, FN = false-negative, FP = false-positive, GC = gastric cancer, HR = hazard ratio, LRN = negative likelihood ratio, LRP = positive likelihood ratio, OS = overall survival, ROC = receiver operating characteristic curves, SROC = summary receiver operator, TN = true-negative, TP = true-positive.

INTRODUCTION

Cancer accounts for the leading cause of mortality in developed countries and the second highest in developing countries, making it a global health issue.[1] It is immediate to diagnose cancer in the early stage by a noninvasive way. Digestive system cancer occupies the most in the cancer incidence and mortality, mainly gastric cancer, colorectal cancer, hepatocellular cancer taken in the top 5.[2,3] As finding molecular targets for digestive system treatment might help to improve the survival of patients with the fatal disease, accumulating studies have attempted to identify biological factors involved in the poor prognosis. However, few molecules have been detected as biomarkers for therapy or diagnosis in clinical application. Therefore, it was significant to search novel biomarkers using a less invasive method.

MicroRNAs (miRNAs), as small and noncoding RNAs, were involved in human carcinogenesis by regulating the translation of specific protein-coding genes. It was supposed that altered expression of miRNAs played an important role in tumorigenesis and the development of various cancers, and it can be stably detectable in plasma/serum.[4–6] Because serum and plasma are relatively easy to acquire, circulating miRNA is one of the most promising candidates for the diagnosis of cancer. MiR-21 was the representative one as it has been largely studied in numerous cancers. The miR-21 is overexpressed in various cancers and has been causally associated to cellular proliferation, apoptosis, and migration.[7] It had been reported that miR-21 induces invasion, intravasation, and metastasis.[8] In the publication of Li's,[9] Zhu's,[10] and Wang's[11] meta-analyses, they separately investigated its specificity and sensitivity as a biomarker in the diagnosis or prognosis of hepatocellular cancer, gastric cancer, and colorectal cancer. Through the discovery of miRNAs and their different expression profiles among different kinds of diseases, the microRNA-21 (miR-21) was the common miRNA which could serve as a potential biomarker for detection for digestive system cancer. It is still existed inconsistencies about diagnostic accuracy of miR-21 though numerous studies investigate the relationship between circulating miRNAs and digestive

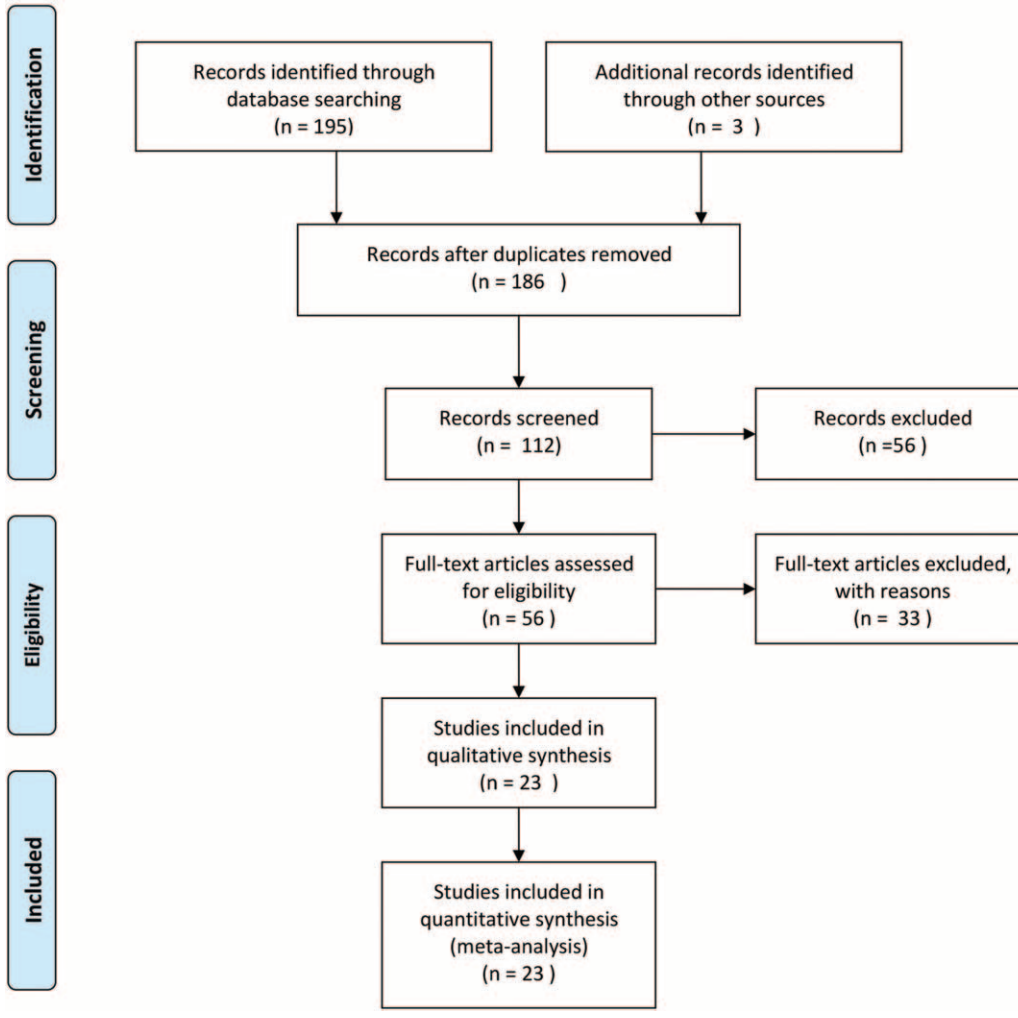

FIGURE 1. Flow diagram of study identification.

system diagnosis. Therefore, based on the whole published related studies, a systematic analysis was performed to evaluate the diagnostic and prognostic efficiency of circulating miR-21 in patients with digestive system cancer.

MATERIALS AND METHODS

Ethical Statement

This is a meta-analysis which focused on the basis of published articles. Ethical statement is not necessary.

Literature Search

Two authors independently searched online PubMed, Embase, and Web of Science up to July 31th, 2015. The keywords used for literature retrieval are "microRNA-21" or "miR-21" or "miRNA-21" or "hsa-miR-21" and "neoplasms[Mesh]" 'and "serum" or "blood" or "plasma." The citations in identified articles and in review articles were also examined. All publications identified by our search strategy were independently assessed by 3 reviewers. Any disagreement on controversial study was resolved by fully discussion to consensus.

Literature Selection

Eligible studies included in this meta-analysis met the following criteria: (1) the diagnosis of any type of digestive system cancer was made based on histopathological confirmation; (2) they detected miR-21 concentration in plasma or serum before patient had treatment; (3) they investigated the association between miR-21 expression levels and digestive system cancer diagnosis or prognosis; and (4) they chose patients with benign disease or healthy people as the control group. In addition, studies exclusion criteria are: (1) review paper and letters; (2) duplicate publications; (3) unqualified data; and (4) non-English publications. All of the literature up to the above criteria is considered to be qualified studies.

Data Extraction and Quality Assessment

The following data characteristics were collected for each included paper: first author, year of publication, country of publication, origin of the study population, sample type, detecting method, sample size, number of participants, follow-up time, and variables adjusted for the analysis. For diagnostic studies, a true-positive (TP), false-positive (FP), false-negative

TABLE 1. The Main Features of Eligible Studies in Diagnostic Systematic Review

Year	Author	Country	Ethnicity	Cancer Type	Case/Control	Sample	AUC	TP	FP	FN	TN
2014	Du M	China	Asia	Colorectal cancer	49/49	plasma	0.877	37	2	12	45
2014	Basati G	Iran	Asia	Colorectal cancer	40/40	serum	0.879	31	9	9	31
2014	Zhang	China	Asia	Colorectal cancer	41/30	plasma	0.657	21	6	20	24
2013	Toiyama	Japan	Asia	Colorectal cancer	186/53	serum	0.927	154	5	32	48
2013	Liu	China	Asia	Colorectal cancer	200/80	serum	0.802	130	12	70	68
2013	Kanana Z	London	Caucasian	Colorectal cancer	20/20	plasma	0.91	18	2	2	18
2012	Wang	China	Asia	Colorectal cancer	32/39	serum	0.85	28	10	4	29
2012	Wang	China	Asia	Esophageal cancer	31/39	serum	0.74	22	12	9	27
2012	Wang	China	Asia	Gastric cancer	30/39	serum	0.81	17	2	13	37
2012	Li	China	Asia	Gastric cancer	70/70	plasma	0.794	52	17	18	53
2011	Zheng	China	Asia	Gastric cancer	53/20	blood	0.853	44	4	9	16
2010	Tsujiura	Japan	Asia	Gastric cancer	69/30	serum	0.673	42	11	27	19
2009	Wang	USA	Caucasian	Pancreatic cancer	49/36	plasma	0.62	23	4	26	32
2013	Tomoya	Japan	Asia	Biliary tract cancer	94/50	plasma	0.93	79	1	15	49
2012	Liu	China	Asia	Hepatocellular cancer	57/59	plasma	0.865	51	17	6	42
2012	Tomimaru	Japan	Asia	Hepatocellular cancer	126/50	plasma	0.953	110	4	16	46
2011	Xu	China	Asia	Hepatocellular cancer	101/90	plasma	0.87	85	25	16	65

AUC = area under the summary ROC curve.

(FN), and true-negative (TN) test result was extracted. For prognostic studies, hazard ratio (HR) estimates with 95% CIs for overall survival (OS) was extracted. If HRs or their 95% CIs were not directly reported in the included studies, they were estimated according to the available survival data by using a method reported by Tierney et al.[12] To assess the quality of the each study included in the diagnostic meta-analysis, the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) checklist which features 14 questions was applied to each article.[13,14] Specifically, each items should be answered with ''yes,'' ''no,'' or ''unclear,'' and only ''yes'' could get a score.

For the studies included in prognostic meta-analysis, quality assessment was performed in each of the acceptable studies in duplicate by independent reviewers (XYZ and YND) on the guidelines of the Newcastle–Ottawa Quality Assessment Scale.[15] Any discrepancies were resolved by a third reviewer (JY).

Statistical Methods

We extracted or calculated the number of patients about TP, FP, TN, and FN from each article for the diagnostic meta-analysis. The bivariate meta-analysis model was used for diagnostic meta-analysis[16] to calculate the sensitivity, specificity, positive likelihood ratio (LRP), negative likelihood ratio (LRN), diagnostic odds ratio (DOR), and generate the bivariate summary receiver operator characteristic (SROC) curve.[17] We examined heterogeneity across studies by using Cochran's-Q and *I*-squared statistics.[18] It was supposed that the studies were homogeneous if the $P > 0.1$ for heterogeneity or *I*-squared $< 50\%$. Deeks' funnel plot asymmetry test was adopted to assess the potential publication bias; it was considered to be a representative of a significant statistical publication bias when *P* value for Deeks' test was <0.1. For the prognostic meta-analysis, HRs and their 95% CIs were used to assess the impact of miR-21 expression on survival of patients with digestive system cancer. All analyses were conducted using stata SE12.0 (Stata Corporation) and Meta-DiSc software.[19]

RESULTS

Literature Search

Searching PUBMED and EMBASE and Web of Science resulted in the inclusion of 174 articles. After a review of titles and abstracts, 68 publications were irrelevant, 13 publications were excluded as review, and 14 publications were excluded as meta-analysis. A total of 48 publications were excluded due to not study of digestive system cancer and 16 publications were excluded due to not covered for diagnosis or prognosis about digestive system cancer or lack of date for analysis. The selection process was shown in Figure 1. Finally, 15 studies were included for diagnosis and 8 articles were included for prognosis. Among the articles for diagnosis, 7 articles were connected with colorectal cancer,[20–25] 4 studies investigated gastric cancer,[23,26–28] esophageal cancer, as well as gastric cancer and colorectal cancer, are investigated in the same article carried out by Wang et al,[23] 3 studies were related to hepatocellular carcinoma,[29–31] 1 study was related to pancreatic cancer,[14] and 1 publication was associated with biliary tract cancer.[32] Among the articles for prognosis, 3 articles were connected with colorectal cancer,[24,25,33] 2 studies investigated gastric cancer,[34,35] 2 studies investigated esophageal cancer,[36,37] and 1 study was related to pancreatic cancer.[38]

Study Characteristics and Quality Assessment

In these 15 qualified articles for diagnosis, there were totally 1248 cases and 716 controls available for this meta-analysis. A total of 13 studies were conducted in Asian and 2 in Caucasian. The sample types were classified as serum (n = 7) and plasma (n = 9). All the 15 studies measured the expression of miRNAs by means of quantitative reverse transcription PCR (qRT-PCR). According to the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool, we systematically evaluated the quality of all the studies. It was demonstrated in Supplementary Table 1, http://links.lww.com/MD/A567 that all the 14 items were replied in each included article. In the 8 eligible articles for prognosis, 7 studies were performed in

TABLE 2. The Main Features of Eligible Studies in Prognostic Systematic Review

Author	Year	Country	Ethnicity	Cancer Type	Number	Sample	HR	Survival	Follow-Up Months	*P*	HR	LL	UL	Quality Score
Menendez	2013	Spain	Caucasian	Colorectal cancer	102	Serum	Reported	OS	0–36	0.071	0.50	0.25	1.02	6
Liu	2013	China	Asia	Colorectal cancer	200	Serum	Reported	OS	4–53	0.126	0.77	3.21	1.5	7
Toiyuma	2013	Japan	Asia	Colorectal cancer	168	Serum	Reported	OS	2–84	0.0049	1.1	15.40	3.21	7
Tanaka	2013	Japan	Asia	Esophageal cancer	64	Serum	DE	OS	0–36	0.4502	0.44	2.76	15.4	7
Komatsu	2012	Japan	Asia	Esophageal cancer	50	Plasma	DE	OS	0–36	0.1038	0.84	6.93	2.76	6
Song	2013	China	Asia	Gastric cancer	103	Serum	DE	OS	24.4–53.1	0.6341	0.46	1.5	6.426	8
Komatsu	2012	Japan	Asia	Gastric cancer	68	Plasma	Reported	OS	0–40	0.0133	1.717	104.42	104.42	7
Liu	2012	China	Asia	Pancreatic cancer	197	Serum	Reported	OS	0–24	<0.001	8.77	2	38.39	7

DE = data-extrapolated, HR = hazard ratio, LL = Lower limit of HR'95% confidence interval, OS = overall survival, UL = Upper limit of HR'95% confidence interval.

Asian and 1 in Caucasian. The sample types contained serum (n = 6) and plasma (n = 2). The characteristics of the included studies were on display in Tables 1 and 2.

Diagnosis Meta-Analysis

Diagnostic Accuracy of Circulating miR-21 in Cancer

The pooled sensitivity and specificity were 0.76 (95% CI = 0.70–0.82) and 0.84 (95% CI = 0.78–0.89), respectively (Fig. 2). And the area under the ROC curve was 0.87(0.84–0.90) (Fig. 3), which indicates miR-21 has a relatively high diagnostic performance in digestive system cancer. Heterogeneity in sensitivity and specificity which were observed among the included studies ($I^2 = 83.35\%$ and $I^2 = 71.24\%$) indicated significant heterogeneity (Fig. 2). It may come from country of origin, type of specimen, sample size, and study quality. In the present studies, the combined LRP is 4.83 (95% CI = 3.43–6.78), which indicates that patients with digestive system cancer have a nearly 5-fold higher chance of being miR-21 test-positive compared with others normal. As to LRN, the combined LRN is 0.28 (95% CI = 0.22–0.37) (Supplementary Figure 1, http://links.lww.com/MD/A566). The heterogeneity analysis shows that the I^2 is 52.93% and 84.04% respectively.

Covariate and Subgroup Analysis

After stratification in accordance with 3 prespecified covariates (ethnicities, sample types, and cancer types), we assess their impact sensitivity or/and specificity through metaregression. We found that studies recruited Asian population enjoys similar sensitivity and specificity (0.77 [95% CI = 0.71–0.82] and 0.84 [95% CI = 0.77–0.89]), separately. The studies of serum sample were also similar to that of plasma in sensitivity (0.72 [95% CI = 0.64–0.80] versus 0.78 [95% CI = 0.68–0.86]) and specificity (0.88 [95% CI = 0.78–0.94] versus 0.87 [95% CI = 0.78–0.92]). In view of many research were focused on the relationship between miR-21 and colorectal cancer, we performed a meta-analysis in colorectal cancer. The pooled results for sensitivity, specificity, LRP, and LRN were 0.76 (95% CI = 66–84%), 0.86 (95% CI = 0.79–0.90), 3.85 (95% CI = 2.50–4.00), and 0.34 (95% CI = 0.25–0.46), respectively. The AUC was 0.89 (95% CI = 0.86–0.91) which implied the circulating miR-21 may have the adequate power to discriminate cancer.

Prognostic Meta-Analysis

Moderate heterogeneity appeared among studies evaluating the correlation between circulating miR-21 expression and OS ($P < 0.05$, $I^2 = 75.2$ %); hence, the random-effect model was used to summarize the pooled HR. According to the final pooled HR of 1.94 (95% CI = 0.99–3.82, $P = 0.055$) (Fig. 4A), it suggested that a higher expression level of miR-21 may conclude worse OS in digestive system cancer. Among the 8 studies, 7 studies recruited patients from the Asian. We then applied a meta-analysis to further explore the potential value of miR-21 in digestive system cancer prognosis in the Asian population. The results demonstrated that the higher miR-21 level was associated with poorer OS (HR = 2.41, 95% CI = 1.21–4.77, $P = 0.012$) (Fig. 4B).

Publication Bias

Begg's funnel plot and Egger's test were performed in the meta-analysis to assess the publication bias in this study. The funnel plots of the diagnostic and prognostic meta-analyses

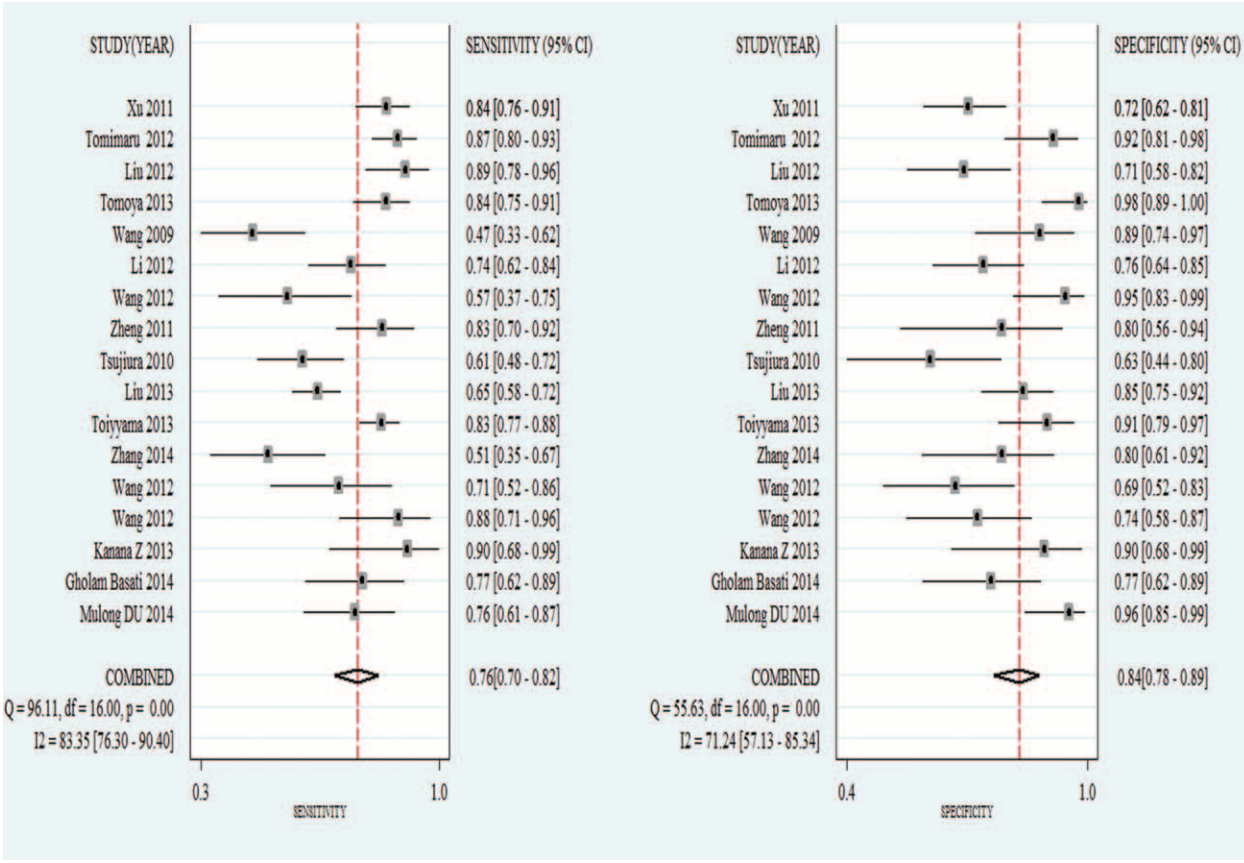

FIGURE 2. Forest plots of sensitivities and specificities from test accuracy studies of miR-21 in the diagnosis of digestive system cancer.

were shown in Figure 5. The *P* value of Begg's test for diagnosis is 0.69 (Fig. 5A). Therefore, there is no sign that publication bias exists. However, as the number of the articles is limited, whether the publication bias exists or not in this meta-analysis is difficult to distinguish. *P* value of Begg's test was 0.013 (Fig. 5B), indicating that there was a publication bias in the meta-analysis for prognosis.

DISCUSSION

Tumor-specific variations in nucleic acids detectable in the plasma are promising noninvasive biomarkers for identifying patients with cancer.[39] When miRNAs were derived from the plasma of cancer patients, they seemed to be more specific and stable in comparison with circulating DNA and mRNA,[40] highlighting its promising use as noninvasive biomarkers for diagnosis and for monitoring various cancers. MiR-21, as one of the most widely studied abnormal miRNAs, was discovered to be upregulated in numerous tumors, such as breast cancer, lung cancer, gastric cancer, colorectal cancer, hepatocellular carcinoma, pancreatic cancer, ovarian carcinoma, and so on. As an oncogene in cells, the molecular mechanism how it adjusts cellular processes had been investigated widely.[41] Aberrant high expression of miR-21 could accelerate cell proliferation, migration, invasion, and survival in vitro cell observation.[11,42] On the contrary, cell proliferation and invasion could be inhibited by means of inducing apoptosis under the knockdown or suppression of miR-21.[7,43,44] Accumulating evidence from retrospective studies manifested that miR-21 was promising to be a biomarker for cancer. A series of quantitative analyses were carried out based on published studies to determine its diagnostic and prognostic value. A meta-analysis by Zeng et al[45] demonstrated that miR-21 was potential to function as a diagnostic biomarker with a moderate sensitivity and specificity for gastric cancer. Wang et al[11] reported in a systematic review and meta-analysis that circulating miR-21 may not be suitable as diagnostic biomarker, but it has a prognostic value in patients with cancer. However, the included articles in this meta-analysis are not complete. Although miR-21 was found to be aberrantly expressed in most cancers and widely studied in tissue or blood, the diagnostic role of circulating miR-21 in various cancers is still a puzzle. Therefore, the purpose was to evaluate the diagnostic role of circulating miR-21 in digestive system cancer by meta-analysis.

After a review of titles and abstracts, 23 studies were up to the standard. We performed the meta-analysis on the basis of the eligible studies. In this meta-analysis, the combined sensitivity and specificity are 0.76 (95% CI = 0.70–0.82) and 0.84 (95% CI = 0.78–0.89), respectively. Glas et al found that when we combine the diagnostic odds ratio (DOR) with the strengths of sensitivity and specificity as prevalence in dependent indicators, the pooled outcome was superior to a single indicator. With the range from 0 to infinity, the higher values of DOR stand for better discriminatory test performance.[46] The DOR value of 17.15 indicates that the miR-21 had potential diagnostic value for GC patients (Supplementary Figure 2,

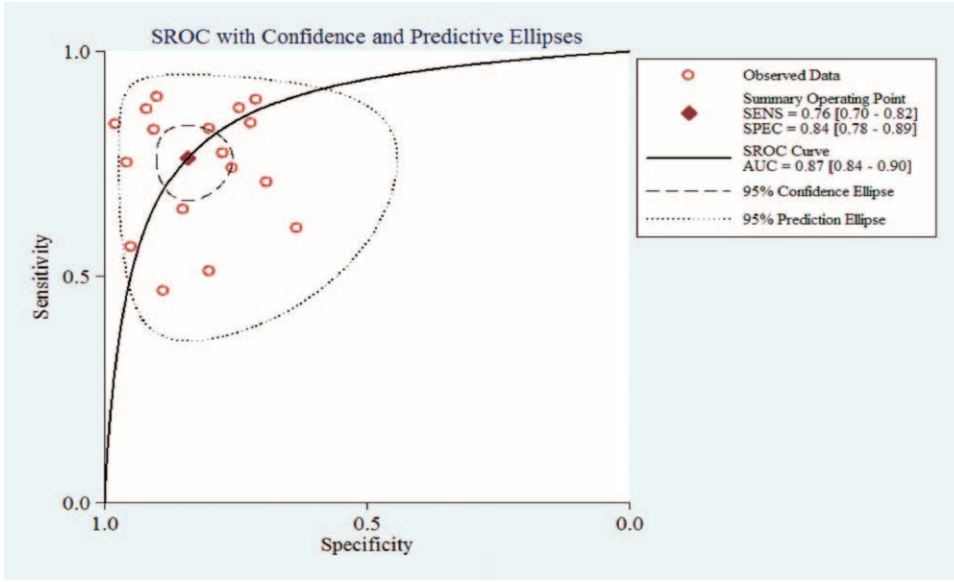

FIGURE 3. Summary receiver operating characteristic curves for miR-21 in the diagnosis of digestive system cancer. The smaller region (confidence contour) contains likely combinations of the mean value of sensitivity and specificity. The wider region (prediction contour) demonstrates more uncertainty as to where the likely values of sensitivity and specificity might occur for individual studies.

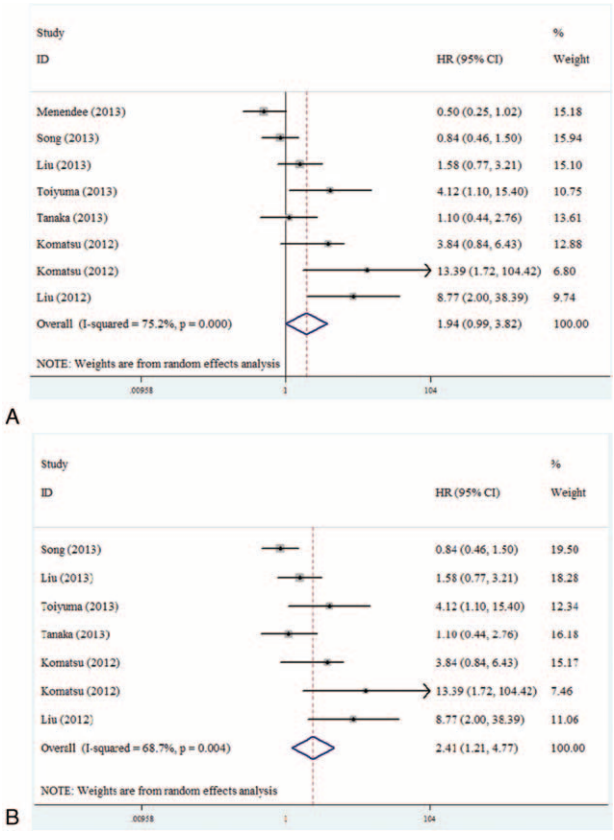

FIGURE 4. Forest plots of studies evaluating the circulating miR-21 expression level and prognosis. (A) Forest plots of OS for all eligible articles. (B) Forrest plots of OS in the Asian. OS = overall survival.

http://links.lww.com/MD/A566). SROC is usually used to sum up overall test performance, and AUC is calculated to evaluate accuracy of the selected indicator. An AUC with the value from 0.93 to 0.96 is considered to be pretty good and 0.75 to 0.92 is acceptable.[47,48] Our studies revealed that miR-21 enjoys good accuracy in the diagnosis of digestive system cancer, with an area under the ROC curve of 0.86. Compared with Wang's result focused on the relation of miR-21 and all cancer types, we had a higher specificity (84% versus 79%) and higher AUC (0.87 versus 0.84) when we focus on the relation of miR-21 and digestive system cancer. It reminded that miR-21 was more suitable for the diagnosis of digestive system cancer. And then we performed subgroup analysis to assess the effect of ethnicities, sample types, and cancer types on sensitivity and specificity. We found that miR-21 in serum have similar sensitivity and specificity with the miR-21in the plasma. So does the ethnicities. We also performed a meta-analysis in colorectal cancer. The pooled results for sensitivity, specificity, LRP, and LRN were 0.76, 0.86, 3.85, and 0.34, respectively (Supplementary Figure 3, http://links.lww.com/MD/A566). The AUC was 0.89 (95% CI = 0.80–0.91) which indicated the circulating miR-21 may have the adequate power either to confirm or to exclude cancer (Supplementary Figure 4, http://links.lww.com/MD/A566).

The results of the prognostic meta-analyses indicated that the circulating miR-21 expression level was a promising biomarker to predict survival in digestive system cancer patients. Patients with an increased level of miR-21 expression had a 1.94-fold higher risk of poor OS and 2.41-fold higher risk of poor OS in the Asians. However, there was significant heterogeneity in the meta-analyses of the data for OS.

Although there were important discoveries revealed by the meta-analysis, there were also some limitations. First of all, most of the controls in diagnostic studies enrolled healthy people and were not blind designed. This design affects the

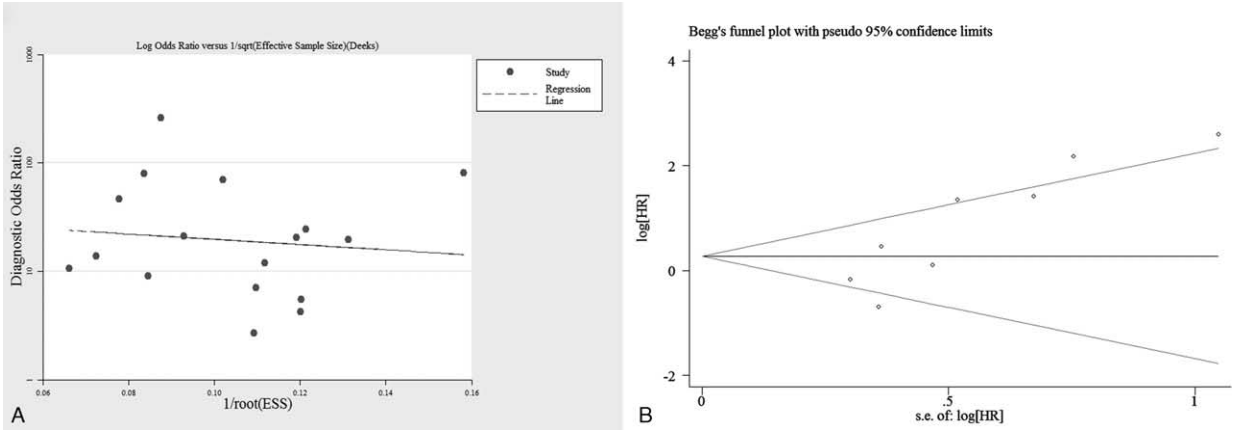

FIGURE 5. Funnel plot for the assessment of potential bias in miR-21 assays. (A) Bias in the diagnosis meta-analysis. (B) Bias in the prognosis meta-analysis.

diagnostic accuracy. Second, considerable heterogeneity existed in this meta-analysis. It was derived from the different detection method of circulating miRNAs among studies. Although subgroup and sensitivity analyses were applied, the results could not fully explain the heterogeneity. Third, the acceptable AUC may not sufficiently specific for gastroenterologic cancer. Fourth, the *P* value of Begg's test for the prognostic meta-analysis bias was 0.013, which meant a publication bias existed in the meta-analysis. The possible reasons may contain population selection bias, different follow-up time, and the sample size. Moreover, only Asians and Caucasians were in the meta-analysis, no African population included in the analysis. Besides, the progression of tumors including sizes, stages, metastasis, and so on seems to largely affect expression levels of miR-21 in the diagnosis of cancer. Other possibilities include other selection biases, true heterogeneity, and data irregularities.

In conclusion, our comprehensive analysis served as a proof-of-concept that the circulating miR-21 expression is a useful noninvasive biomarker for the early detection of digestive system cancer and promising marker for digestive system cancer prognosis in the Asian population. Even so, further large-scale prospective studies are warranted to confirm our analysis.

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